In vitro activities of ceftazidime/avibactam alone or in combination with fosfomycin against carbapenem-susceptible and -resistant *Klebsiella pneumoniae* strains isolated from Intensive Care Units

E. Mataracı Kara and **B. Özbek Çelik**

Department of Pharmaceutical Microbiology, Faculty of Pharmacy, Istanbul University, 34116, Beyazıt/Istanbul, Turkey

Aims: This study aim to analysis the effect of ceftazidim/avibactam, fosfomycin alone and ceftazidim/avibactam+fosfomycin combination against carbapenem-susceptible and -resistant *Klebsiella pneumoniae* strains.

Methods: Fifty clinical strains were screened for their MICs of carbapenems, ceftazidime/avibactam and fosfomycin and MICs were determined by microbroth dilution method. Also, the in vitro synergistic activities of ceftazidime/avibactam+fosfomycin combination were determined by time-kill curve assays both at 0,5xMIC and at 1x MIC against two carbapenem-susceptible and three carbapenem-resistant *K. pneumoniae* isolates.

Results: Based on MIC results, 88% of the 50 isolates of *K. pneumoniae* were resistant to fosfomycin, 86% resistant to carbapenems, and 50% resistant to ceftazidime/avibactam. Results of time-kill curve assay showed that ceftazidime/avibactam+fosfomycin combination was bactericidal against two strains and it was also additive for all tested strains both at 0.5xMIC and 1xMIC.

Conclusion: The findings of this study suggest that ceftazidime/avibactam+fosfomycin combination was additive against both carbapenem-susceptible and -resistance *K. pneumoniae* strains. In our opinion, this combination might be useful to inhibit developing resistance than antibiotics used alone.

Keywords: *Klebsiella pneumoniae*; combination.

1. Introduction

Klebsiella pneumoniae is one of the most important causes of health care associated infections, including pneumonia, bloodstream infections, wound or surgical site infections, and meningitis [1, 2]. The emergence and global spread of infections caused by carbapenem-resistant *K. pneumoniae* are of a great concern worldwide because they are associated with high mortality rates [1, 2, 3]. Data from retrospective studies have concluded that combination antibiotic therapy is associated with a better outcome than monotherapy for the treatment of severe infections with carbapenem-resistant *K. pneumoniae*. [2]. Due to the limited treatment option, thus, interest in its effectiveness against MDR or XDR nosocomial infections, when limited treatment options are available, fosfomycin has been reawakened [3]. Besides, ceftazidime/avibactam is a novel beta lactam/beta lactamase inhibitor combination recently approved by the FDA for treatment of complicated urinary tract infection, complicated intra-abdominal infection in combination with metronidazole and also for treatment of hospital acquired pneumoniae including ventilator-associated pneumoniae caused by Enterobacteriaceae and *Pseudomonas aeruginosa* [4]. However, inadequate data exist on whether ceftazidime/avibactam+fosfomycin combination is effective, especially even if the isolated bacteria are susceptible or resistant to carbapenems. Therefore, the present study aimed to identify the antimicrobial effect of ceftazidime/avibactam and fosfomycin alone or in combination against carbapenem-susceptible and –resistant *K. pneumoniae* strains isolated from intensive care units.

2. Materials and Methods

2.1 Bacterial strains

Fifty non-duplicate, nosocomially-acquired carbapenem-susceptible and –resistant *K. pneumoniae* isolates were randomly collected from the Department of Infectious Diseases and Clinical Microbiology at an intensive care unit of a tertiary care center in Istanbul at the first six month of 2016. All strains were identified using API 20E (bioMérieux, Marcy-l'Étoile). As a reference strain, *E. coli* ATCC 25922 and *K. pneumoniae* ATCC 700603 (American Type Culture Collection, Rockville, MD., USA) were used throughout the study. Two carbapenem-susceptible and three carbapenem-resistant *K. pneumoniae* isolates were chosen for the in vitro synergistic activities of ceftazidime/avibactam+fosfomycin combination which were determined by time-kill curve assay both at 0,5xMIC and at 1xMIC concentrations.

2.2 Antibiotics

All antimicrobial agents were kindly provided by their respective manufacturers. Stock solutions of fosfomycin and ceftazidime/avibactam were stored frozen at −80°C. Frozen solutions of antibiotics were used within six months. Carbapenems (doripenem, imipenem, meropenem, and ertapenem) solutions were prepared on the day of use.

2.3 Media

Mueller-Hinton broth (Difco Laboratories, Detroit, MI, USA) was used for MIC, and time-kill curve studies, and supplemented with 25mg of calcium/liter and 12.5mg of magnesium/liter (CAMHB). Pour plates of Tryptic soy agar (Difco Laboratories) were used for colony counts.

2.4 MIC Determinations

MICs were determined by the microbroth dilution technique described by CLSI quideline [5]. Serial two-fold dilutions ranging from 256 to 0.125 mg/L for studied antibiotics were prepared in CSMHB 96-well microtiter plates. The MIC was defined as the lowest concentration of antibiotic giving complete inhibition of visible growth.

2.5 Time-kill Curve Studies

Time–kill curve assays were performed on five isolates representing two distinctive with different susceptibility patterns. To evaluate concentration-dependent synergistic interaction, these strains were exposed to ceftazidime/avibactam+fosfomycin combination at 0,5x and 1x the MIC, following methods published by the NCCLS [6]. The lower limit of detection by this method was 10 CFU/ml. Synergy and antagonism were defined as ≥ 2 $\log_{10}$ decrease or increase, respectively, in CFU/ml at 24 h for the antibiotic combination when compared with its more active constituent. Additive was defined as a < 2 $\log_{10}$ change (increase or decrease) in colony count at 24 hours by the combination, in comparison with the most active single antimicrobial alone.

3. Results

The MICs of the antimicrobial agents against 50 clinical isolates of *K. pneumoniae* are summarized in Table 1. Moreover, 88% of the studied strains were resistant to fosfomycin ($\geq$ 256 mg/L), 86% resistant to carbapenems, and 50% resistant to ceftazidime-avibactam. On the other hand, in our study, 46.8% (22 of 47 strains) of carbapenem-resistant strains were found to be susceptible to ceftazidime/avibactam. All of the carbapenem-susceptible strains were also found to be susceptible to ceftazidime/avibactam.

The selected strains for time-kill curve assay have been exhibited different susceptibility patterns for ceftazidime/avibactam and carbapenems. KP-1 and KP-5 were resistant to all tested antibiotics, KP-2 was only susceptible to ceftazidime/avibactam, KP-3 and KP-4 were susceptible for both ceftazidime/avibactam and carbapenems. However, time-kill curve assays revealed that there are only minor differences in the kinetics of tested combination, at 1xMIC our combination achieved bactericidal effect to our two ceftazidime/avibactam-susceptible and carbapenem-susceptible strains (KP-3 and KP-4, Fig. 1). Only additive effect was observed in combinations of ceftazidime/avibactam with fosfomycin against carbapenem-resistant and carbepenem-susceptible strains or the ceftazidime/avibactam-resistant and ceftazidime/avibactam-susceptible strains. No synergism or antagonism were seen.

4. Discussion

Carbapenem-resistant *K. pneumoniae* is an important therat to global health [1]. This is likely due in part to limited treatment options which include polymyxins, tigecycline [2]. Nephrotoxicity is the major adverse event associated with the use of polymyxins. Also, low blood levels limit the use of tigecycline [2, 7]. However, fosfomycin, is a useful agent for the treatment of MDR and XDR nosocomial infections when limited treatment options are available. Since, fosfomycin has been associated with the rapid development of resistance in vitro, widespread or increasing resistance in clinical practice has been infrequently reported [3, 8]. Similarly to previous studies, in the present research, 88% of the tested *K. pnuemoniae* strains displayed resistant to fosfomycin. Although our research displayed that approximately half of the carbapenem-resistant strains were found to be susceptible to ceftazidime/avibactam, all of the carbapenem-susceptible strains were found to be susceptible to ceftazidime/avibactam. It could be revealed that the importance of the ceftazidime/avibactam on the therapy of infection caused by *K. pneumoniae*.

Data from retrospective comparisons favor combination therapy over single-agent therapy, with absolute differences in mortality ranging from 20.2% to 46.7% against carbapenem-resistant Enterobacteriaceae bloodstream infections [10]. Moreover, combination therapy has been recommended not only to combat carbapenem-resistant *K. pneumoniae* infections but also to inhibit or reduce the emergence of resistance during treatment [9, 10, 13]. In the same time, synergy was reported when fosfomycin used combination treatment with other antibiotics was revealed both in clinics and in vitro

[9, 10, 11]. Ceftazidime/avibactam is a novel drug combination that could be useful in some cases of difficult to treat Gram negative infections, when there are few or no therapeutic options [12, 14, 15]. Therefore, the present study, we aimed to analyse the in vitro synergistic activity of ceftazidime/avibactam and fosfomycin combination against carbapenem-susceptible and -resistant *K. pneumoniae* isolates from intensive care units at 0,5x and 1xMIC. Parallel to a previous study by Falcone et al. [12] were shown that the effective bacterial killing of ceftazidime/avibactam against Enterobacteriaceae including *K pneumoniae*, we could determined bactericidal effects at 1xMIC against our carbapenem- and ceftazidime/avibactam susceptible *K. pneumoniae* (Fig. 1- KP-3 and KP-4). Although, the combination of ceftazidime/avibactam with imipenem has been reported to have synergistic activity against KPC-*K. pneumoniae* isolates [16], in our study the combination of ceftazidime/avibactam with fosfomycin has only shown additive with both the 0,5x and 1xMIC used.

In conclusion, our research shown that approximately half of the carbapenem-resistant strains were found to be susceptible to ceftazidime/avibactam. All of the carbapenem-susceptible strains were also found to be susceptible to ceftazidime/avibactam. Also, in our study, two ceftazidime/avibactam and carbapenem-susceptible strains were shown bactericidal effect at 1xMIC. Ceftazidime/avibactam+fosfomycin combination showed additive against both carbapenem-susceptible and -resistant *K. pneumoniae* isolates. It is might be estimated that carbapenem-resistance and ceftazidime/avibactam-resistant do not significantly influence the combination interaction.

In our opinion, the combination is more warranted in order to lowering their potential side effects and preventing the development of resistance. The findings of this study may have important information for the optimal combination to combat the carbapenem-resistant *K. pneumoniae* infections. More studies are required to confirm the efficacy and to evaluate the clinical use of this combination.

Acknowledgements This work was supported by a grant from the Research Fund of the University of Istanbul. Project number: 21897.

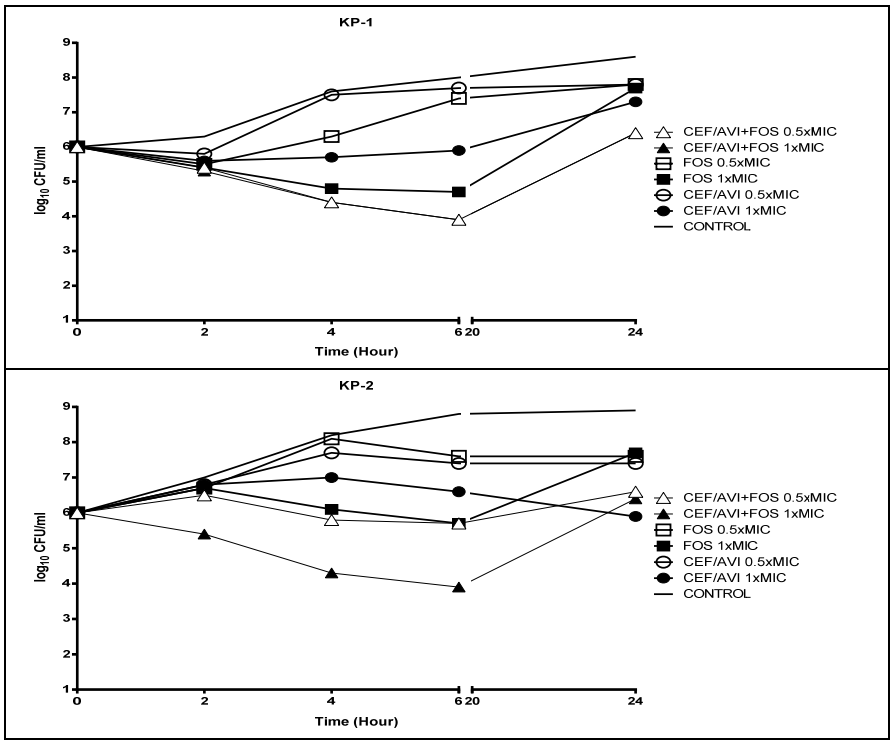
References

[1] Codjoe FS, Donkor ES. Carbapenem Resistance: A Review. Med Sci 2018;6(1):1-28.
[2] Petrosillo N, Giannella M, Lewis R, Viale P. Treatment of carbapenem-resistant Klebsiella pneumoniae: the state of the art. Expert Review of Anti-infective Therapy, 2013;11:2, 159-177.
[3] Falagas ME, Vouloumanou EK, Samonis G, Vardakasa KZ. Fosfomycin. Clin Microbiol Rev 2016; 29:321–347.
[4] Sader HS, Castanheira M, Flamm RK, Farrell DJ, Jones RN. 2014. Antimicrobial activity of ceftazidime-avibactam against gram-negative organisms collected from U.S. medical centers in 2012. Antimicrob Agents Chemother. 58:1684–1692.
[5] Clinical and Laboratory Standards Institute: Methods for Dilution antimicrobial susceptibility tests for bacteria that grow aerobically: Approved standard M7-A7, ed 7, Wayne, CLSI, 2006.
[6] National Committee for Clinical Laboratory Standards: Methods for Determining Bactericidal Activity of Antimicrobial Agents-Approved Guideline M26 A. Wayne NCCLS, 1999.
[7] Pournaras S, Vrioni G, Neou E, Dendrinos J, Dimitroulia E, Poulou A, Tsakris A. Activity of tygecycline alone and in combination with colistin and meropenem against Klebsiella pneumoniae carbapenemase (KPC)-producing Enterobacteriaceae stains by time-kill assay. Int J Antimicrob Agents 2011;37:244-247.
[8] Vardakas KZ, Legakis NJ, Triarides N, Falagas ME. Susceptibility of contemporary isolates to fosfomycin: a systemetic eview oft he literature. Int J Antimicrob Agents 2016;47: 269-285.
[9] Girometti N, Lewis RE, Gianella M, Ambretti S, Bartoletti M, Tedeschi S, Tumietto F, Cristini F, Trapani F, Gaibani P, Viale P. Klebsiella pneumoniae bloodstream infection. Medicine 2014;93(17): 298-308.
[10] von Duin D, Kaye KS, Neuner EA, Bonomo RA. Carbapenem-resistant Enterobacteriaceae: a review of treatment and outcomes. Diagn Microbiol Infect Dis 2013;75: 115-120.
[11] Olivia A, Cipolla A, Gizzi F, D'Abramo A, Favaro M, de Angelis M, Ferretti G, Russo G, Iannetta M, Mastroianni CM, Mascellino MT, Vullo V. Severe bloodstream infection due to KPC- producer E. coli in a renal transplant recipient treated with the double-carbapenem regimen and analysis of in vitro synergy testing. Medicine 2016;95(7):1-5.
[12] Falcone M, Paterson D. Spotlight on ceftazidime/avibactam: a new option for MDR Gram negative infections. J Antimicrob Chemother 2016;17: 1-10.
[13] Rapp RP, Urban C. Klebsiella pneumoniae carbapenemases in Enterobacteriaceae: history, evolutıon, and microbiology concerns. Pharmacotherapy 2012;32(5): 399-407.
[14] Duin D, Bonomo RA. Ceftazidime/Avibactam and Ceftolozane/Tazobactam: Second-generation β-Lactam/β-Lactamase Inhibitor Combinations. CID 2016; 63(2): 234–41.
[15] Sader HS, Castanheira M, Flamm RK, Farrell DJ, Jones RN. Antimicrobial activity of ceftazidime-avibactam against gram-negative organisms collected from U.S. medical centers in 2012. Antimicrob Agents Chemother 2014; 58:1684–92.
[16] Gaibani P, Lewis RE, Volpe SL, Giannella M, Campoli C, Landini MP, Viale P, Re MC, Ambretti S. In vitro interaction of ceftazidime–avibactam in combination with different antimicrobials against KPC-producing *Klebsiella pneumoniae* clinical isolates. Int J Infect Dis, 2017;65:1-3.

Table 1 In vitro activities of ceftazidime/avibactam, fosfomycin and carbapenems against 50 clinically obtained strains of *Klebsiella pneumoniae*.

Antibiotics	MIC (mg/L)			Susceptibility (n (%))		
	MIC range	MIC_{50}	MIC_{90}	S	I	R
CEF/AVI	0,25- > 256	8	>256	25 (50)	0 (0)	25 (50)
FOS	128- >256	256	>256	0 (0)	6 (12)	44 (88)
Carbapenems						
ETP	0,25- >256	256	>256	0 (0)	1 (2)	49 (98)
IPM	0,5- >256	64	256	3 (6)	0 (0)	47 (94)
MEM	0,25- >256	64	256	7 (14)	0 (0)	43 (86)
DOR	0,25- >256	32	256	6 (12)	0 (0)	44 (88)

CLSI breakpoints for susceptibility and resistance to ceftazidime/avibactam (CEF/AVI) are ≤8/4 mg/L, and ≥ 16 mg/L, respectively and for susceptibility and resistance to fosfomycin (FOS) are ≤ 64 mg/L, and ≥ 256 mg/L, respectively and for susceptibility and resistance to ertapenem (ETP) are ≤ 0.5 mg/L, and ≥ 2 mg/L, respectively, for susceptibility and resistance to imipenem (IPM) are ≤ 1 mg/L, and ≥ 4 mg/L, respectively, for susceptibility and resistance to meropenem (MEM) are ≤ 4 mg/L, and ≥ 16 mg/L, respectively, and for susceptibility and resistance to doripenem (DOR) are ≤ 1 mg/L, and ≥ 4 mg/L,respectively.



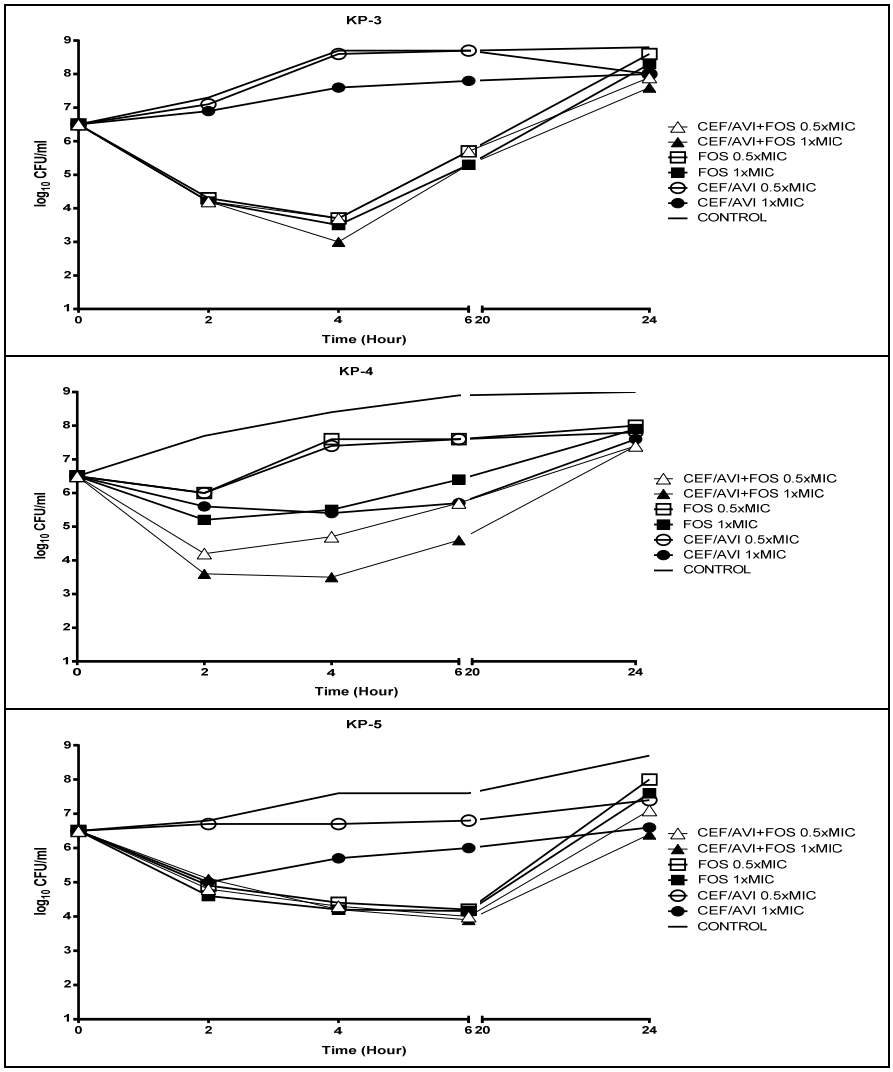


Fig. 1 Time-kill curve determinations for two carbapenem-susceptible and three carbapenem-resistant *K. pneumoniae* strains after treatment with ceftazidim/avibactam (CEF/AVI) alone or in combination with fosfomycin (FOS) at 0.5 x MIC or 1 x MIC. The x-axis represents the killing time, and the y-axis represents the logarithmic carbapenem-susceptible and –resistant *K.pneumoniae* survival.